CF mutant and wild type mice were maintained under standard conditions except that Colyte was substituted for drinking water. Substitution of drinking water with Colyte (an electrolyte solution containing 6% polyethylene glycol) has been shown to allow certain CF mutant mice to consume mouse food, which assists in prolonging their life span and has certain advantages over a liquid diet (Grubb, B. R., *Am. J. Physiol.* 268: G505-G513, 1995.)

Wild type and CF mice were exposed to a humidified atmosphere (produced as described in Example 4) containing 1 μM thapsigargin for 3 hours/day for 7-14 days. For histologic examination of lung tissue, wild type animals exposed to this treatment for 21 days revealed no gross pathologic changes (Figure 8). The night before the NPD procedure was performed, mice were taken off Colyte and given water or alimentum (liquid formula). Wet food was also withheld. These steps were taken to decrease the risk of dehydration and/or intestinal obstruction that occur with sedation and dehydration.

The NPD protocol was performed as follows:

A 10 ml syringe was filled with each test solution, making sure that there were no bubbles in the microperfusion pump system. The 4 solutions used were: i) control-Ringers, ii) Ringers with amiloride 10^{-5} M, iii) Ringers with 0mM chloride and amiloride 10^{-5} M, iv) Ringers with 0mM chloride, amiloride 10^{-5} M, and isoproterenol 10^{-5} M. The electrodes were then attached to a voltmeter. One electrode was used as a subcutaneous reference electrode (27 gauge butterfly needle placed either in the belly or the tail) and the other was included in the system leading to the nose. Before attaching the electrodes to the system, agar bridges were placed in control Ringer solution, and the electrodes were zeroed. The syringe pump was set to recognize 10 ml syringes, and the flow rate was set to 0.15 mls/hr.

Mice were anaesthetized with Ketamine 100mg/kg (range 75-100 mg/kg) and Xilazine 10 mg/kg (range 5-10mg/kg) (ketamine and xilazine were either prediluted with saline and then mixed in a 1 ml syringe or were mixed in a microfuge tube with micropipetters and then diluted with saline). A total volume of 0.5mls was used for intraperitoneal injection into the right side of the lower abdomen. If second dosages of anaesthesia were needed intraperitoneal injection of 50mg/kg ketamine and 5mg/kg xilazine (0.5 ml total volume) was performed.

A heat pad was warmed in a microwave oven for 1 min and then for a further 30 secs to achieve an appropriate temperature for maintaining mice during the NPD procedure. Each mouse was placed on a heat pad and PE10 tubing inserted into the nose.

The end of the tubing was previously pulled to a very small diameter under the microscope to minimize trauma to mouse nasal mucosa. Saline eye drops were applied intermittently to decrease risk of corneal abrasions during the procedure.

After obtaining a stable baseline reading, infusion of Ringers solution was begun, and recording was initiated. After 5 mins of stable reading, the solution was changed successively to: (i) Ringer solution containing amiloride 10^{-5} M; (ii) Ringer solution containing 0 mM chloride and 10^{-5} M amiloride; (iii) Ringer solution containing 0 mM chloride, 10^{-5} M amiloride, and 10^{-5} M isoproterenol. NPD was recorded for each solution for 5 minutes of stable values. Following the procedure 1cc of warm saline was injected IP to aid with rehydration. After recovery mice were maintained on a liquid diet overnight.

Each data point in Figure 7 represents an average of results obtained using groups of 4 - 6 animals. Error bars represent standard error. Statistical analysis was performed using Jandel's Sigmastat and Excel.

Results. In human CF patients, both upper and lower airways exhibit reduced or absent cAMP-mediated Cl^- secretion and hyperabsorption of Na^+. It is believed that the hyperabsorption of Na^+ and osmotically linked water absorption contribute substantially to the thick, viscous mucus that characterizes the disease. In humans with CF, measurement of the electrical potential across the nasal mucosa *in vivo* has been used to demonstrate hyperabsorption of Na^+ across the airway epithelium (Knowles, M., Gatzy, J., and Boucher, J., "Increased bioelectric potential difference across respiratory epithelia in cystic fibrosis", *N. Engl. J. Med.* 305: 1489-1495, 1981). The same technique has been applied to the mouse. CF patients and various CF mouse models in which the murine CFTR gene has been mutated, deleted, or replaced by a mutant CFTR gene containing a mutation corresponding to a CF-causing mutation in humans (referred to herein as CF mice) exhibit a raised (i.e., more negative) baseline transnasal potential difference (NPD) as compared to that in normal subjects (Grubb, B.R., et al., Inefficient gene transfer by adenovirus vector to cystic fibrosis airway epithelia of mice and humans, *Nature*, 371: 802-806, 1994; Grubb, B.R., Vick, R. N., and Boucher, R.C., *Am. J. Physiol.*,266: C1478-1483, 1994; reviewed in Grubb, B. and Boucher, R.C., Pathophysiology of gene-targeted mouse models for cystic fibrosis, *Physiological Reviews*, 79 (Suppl 1), 1999). Furthermore, various CF mice display a significantly greater decrease in NPD in response to amiloride, a drug that blocks electrogenic Na^+ absorption, than do control mice. In normal mice and humans perfusion of the nasal mucosa with a solution containing a low Cl^- concentration leads to a hyperpolarization of the NPD. In contrast, in CF individuals either no change or a slight

depolarization of the basal PD is observed under such conditions. Thus the alterations in NPD that characterize CF mice appear to accurately reflect those seen in human CF subjects. These results suggest that treatments tending to restore the behavior of the NPD in CF mice towards that observed in normal mice will have similar effects in human CF patients and are likely to be effective treatments for CF.

To determine the effect of thapsigargin treatment *in vivo*, we measured nasal potential difference (NPD) in thapsigargin treated or untreated wild type and genotypically CF mice. The transnasal potential difference (NPD) reports the electrical potential difference across the nasal epithelial cells, and thus permits the assessment of these cells' capacity to participate in absorption and secretion of Na^+ and Cl^-.

As can be seen in Figure 7, treated (open squares) and untreated (filled squares) wild type animals manifest a small lumen negative transepithelial potential that is further reduced by the addition of the sodium channel blocker amiloride. Replacement of the fluid in the lumen with a solution containing 0 mM Cl^- results in increases in the magnitude of the lumen negative potential. This effect is further enhanced through the addition of isoproterenol, which stimulates CFTR by raising intracellular cAMP levels.

These results are consistent with the interpretation that, in normal mice (and humans), the nasal epithelium carries out electrogenic Na^+ absorption, mediated by an amiloride-sensitive Na^+ channel. The presence of the CFTR chloride channel on the apical surfaces of these cells allows Cl^- to follow Na^+ and thus reduces the magnitude of the transepithelial potential. In the presence of amiloride and in the absence of lumenal Cl^- CFTR permits net Cl^- secretion, which is further stimulated by activation of CFTR through isoproterenol treatment. Mice homozygous for a CF-causing mutation (open circles) exhibit a markedly increased amiloride-sensitive lumen negative potential, consistent with the absence of a conductive pathway for Cl^-. Similarly, removal of lumen Cl^- and isoproterenol treatment do not enhance net Cl^- secretion in CF mice. In thapsigargin-treated CF mice (filled circles), the NPD is markedly reduced relative to that in untreated CF mice ($p < 0.05$), approximating that seen in wild type mice. Normal levels of net Cl^- secretion are detected in CF mice that have been treated with thapsigargin when lumen Cl^- is removed in the presence of amiloride and isoproterenol, whereas untreated CF mice exhibit a markedly reduced (i.e., less negative) NPD ($p < 0.05$).

All of the animals tolerated thapsigargin treatment without exhibiting any obvious morbidity. Figure 8 shows the histologic appearance of lung tissue from control mice and mice treated with thapsigargin for 21 days. Panel A shows sections of lung tissue from